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FORAMEN OVALE BLOOD FLOW AND CARDIAC FUNCTION AFTER MAIN PULMONARY ARTERY OCCLUSION IN FETAL SHEEP

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Subject area**New findings:**

- **What is the central question of this study?**

At near term gestation, foramen ovale blood flow accounts for a significant proportion of fetal left ventricular output. Can foramen ovale increase its volume blood flow, when right ventricular afterload is increased by main pulmonary artery occlusion?

- **What is the main finding and its importance?**

Foramen ovale volume blood flow increased during main pulmonary artery occlusion. However, this increase was attributable to a rise in fetal heart rate, because left ventricular stroke volume remained unchanged. These findings suggest that foramen ovale has a limited capacity to increase its volume blood flow.

Abstract

Foramen ovale (FO) accounts for the majority of fetal left ventricular (LV) output. Increased right ventricular (RV) afterload can cause a redistribution of combined cardiac output between the ventricles. To understand the capability of FO to increase its volume blood flow and thus LV output, we mechanically occluded the main pulmonary artery in seven chronically instrumented near term sheep fetuses. We hypothesised that FO volume blood flow and LV output would increase during main pulmonary artery occlusion. Fetal cardiac function and haemodynamics were assessed by pulsed and tissue Doppler at baseline, 15 and 60 min after occlusion of the main pulmonary artery and 15 min after occlusion was released. Fetal ascending aorta and central venous pressures, and blood gas values were monitored. Main pulmonary artery occlusion initially increased fetal heart rate ($p<0.05$) from 158(7) to 188(23) bpm and LVCO ($p<0.0001$) from 629(198) to 776(283) ml/min. Combined cardiac output fell ($p<0.0001$) from 1524(341) to 720(273) ml/min. During main pulmonary artery occlusion, FO volume blood flow increased ($p<0.001$) from 507(181) to 776(ml/min). This increase was related to fetal tachycardia, because LV stroke volume did not change. Fetal ascending aorta blood pressure remained stable. Central venous pressure was higher ($p<0.05$) during the occlusion than after it was released. During the occlusion fetal pH decreased and $p\text{CO}_2$ increased. LV systolic dysfunction developed while LV diastolic function was preserved. RV systolic and diastolic function deteriorated following the occlusion. In conclusion, FO has a limited capacity to increase its volume blood flow at near term gestation.

Introduction

Fetal right (RV) and left (LV) ventricles pump in parallel into the systemic circulation. The RV is mainly responsible for lower body and placental blood flow and perfusion while myocardial, brain and upper body blood flow is provided by the LV. Under physiologic conditions in near term fetal sheep, about 70% of combined cardiac output (CCO) is ejected by the right ventricle (RVCO), and almost 90% of RVCO is directed through the ductus arteriosus (DA) towards the lower body and placenta, and the rest to the pulmonary circulation (Anderson, Bissonnette, Faber & Thornburg, 1981; Rudolph, 1985). Since fetal pulmonary circulation is under acquired vasoconstriction at near term gestation (Lewis, Heymann & Rudolph, 1976), blood flow across the foramen ovale (FO) accounts for 34 % of CCO and 85 % of LV output (LVCO) (Anderson et al., 1981). However, changes in ventricular loading conditions can lead to redistribution of CCO between the two ventricles and disturb the RV dominance as RVCO is particularly sensitive to increased afterload (Reller, Morton, Reid & Thornburg, 1987; Thornburg & Morton, 1983). During prolonged DA occlusion in near term fetal sheep, RVCO and CCO decreased while LVCO increased (Hashima et al., 2015). Interestingly, a rise in LVCO was attributable to increased pulmonary volume blood flow while FO volume blood flow did not change (Hashima et al., 2015). This suggests that at near term gestation fetal pulmonary circulation is an important regulator of LVCO whereas FO blood flow may be at or near its maximum capacity.

To investigate the capability of fetal FO to increase its volume blood flow and thus improve LV filling and output, we performed a complete mechanical main pulmonary artery occlusion in chronically instrumented near term fetal sheep. During the occlusion, there is no forward blood flow across the DA and the LV alone accounts for systemic and placental circulation. Furthermore, LV preload is preferentially supplied by the FO blood flow. We hypothesised that FO volume blood flow, and hence LVCO, would increase during main pulmonary artery

occlusion in order to maintain adequate systemic and placental perfusion. The specific aims of this study were to investigate the effect of main pulmonary artery occlusion on fetal 1) LVCO, CCO and systemic arterial and central venous pressures, 2) RV and LV systolic and diastolic functions, 3) peripheral venous blood flow patterns, and 4) placental volume blood flow and fetal oxygenation.

Material and Methods

Ethical Approval

The study protocol was approved by the National Animal Experiment Board of Finland (ESAVI/3510/04.10.03/2011). The animal transport, husbandry and experimental procedures were performed in compliance with the national legislation (Act and Decree on the protection of animals used for scientific or educational purposes) and the EU directive 2010/63/EU. The investigators acknowledge the ethical principles of *Experimental Physiology*, and confirm that the study was conducted in compliance with animal ethics checklist (Grundy, 2015). Altogether seven time mated pregnant 1-7 years old Aland landrace sheep weighing between 41-53 kg (Lammastila Sikka Talu, University of Turku, Rymättylä, Finland) were used in the experiment. The sheep were transported from the breeder to the Laboratory Animal Centre at the University of Oulu, Finland two weeks before the experiment. During the adaptation period, the sheep were group housed in two pens of 10.8 m² in area and during the experiment in individual pens of 3.6 m², with straw bedding. Adjacent sheep were able to be in contact with each other through the windows between the pen walls, and no individual sheep was left alone in the animal room. The room temperature was 18 ±2°C, ventilation rate 15 times/hour and humidity 45±5 %. The light-dark cycle was 12:12 with the lights off at 6 pm. The sheep were given tap water and hay *ad libitum* and they had a salt block in the pen. Individually rationed oat grains, turnip rape based protein supplement (Farmarin rypsi, Hankkija-Maatalous Oy, Seinäjoki, Finland) and mineral and vitamin supplement (Lammas Hertta, Hankkija-Maatalous Oy, Seinäjoki, Finland) were given twice daily and the rations were increased gradually

towards the end of the pregnancy. When needed, supportive doses of calcium were given either orally or intravenously. Animals were monitored several times daily by a veterinarian, animal technicians and the investigators for signs of pain, distress, injury or disease. The focus was set to ensure the well-being of animals and to minimize pain and suffering (see methodological description below).

Surgical protocol

The sheep with either singleton or twin pregnancies were operated on at 120-130 gestational days (term 145 days). In case of twin pregnancy, only one fetus was instrumented. The sheep were premedicated with intramuscular ketamine (2 mg/kg, Ketaminol vet, Intervet, Boxmeer, Netherlands) and midazolam (0.2mg/kg, Midazolam Hameln, Hameln Pharmaceuticals gmbh, Hameln, Germany). The left external jugular vein was cannulated for intravenous access and Ringer's lactate solution was infused at a rate of 200ml/h. General anaesthesia was induced with intravenous propofol (4-7mg/kg, Propofol-Lipuro, Braun, Melsungen, Germany) and maintained with isoflurane (1.5-2.5%, Isofluran Baxter, Baxter S.A., Lessines, Belgium) in an oxygen-air mixture delivered via an endotracheal tube. Mechanical ventilation was provided with a Siemens 730 ventilator (Siemens-Elima AB, Solna, Sweden). Maternal heart rate and arterial blood pressure were invasively monitored via a cannulated auricular artery. For pain relief, intravenous boluses of fentanyl (0.05-0.15 mg, Fentanyl-Hameln, Hameln Pharma plus gmbh, Hameln, Germany) were administered on the basis of changes in maternal heart rate and arterial blood pressure during surgical stimuli as deemed necessary by an experienced anaesthesiologist.

A midline abdominal incision was made to access the uterus. The fetal head and upper body were delivered. Polyvinyl catheters were inserted into the carotid artery and internal jugular vein, with the catheter tips in the ascending aorta and superior vena cava. A left lateral thoracotomy was performed and the pericardial sac was opened to expose the great arteries. The main pulmonary artery was isolated and a 6mm vascular occluder (In Vivo Metric, Healdsburg, CA, USA) was placed

around it between pulmonary valve and the bifurcation of left and right pulmonary arteries (Figure 1). A 3-lead 28-gauge silver coated copper electrocardiogram wire (New England Wire Tech., Lisbon, NH, USA) was placed subcutaneously on the fetal chest. Thereafter, the fetal chest was closed. A separate polyvinyl catheter was placed in the amniotic cavity. Lost amniotic fluid was replaced with warm saline solution and an intra-amniotic injection of penicillin G (1 million Units, Geopenil, Orion Oyj, Espoo, Finland) was administered. The surgical incisions were closed. All catheters were tunnelled subcutaneously and exteriorized through a small incision in the ewe's flank. Postoperative analgesia was provided with transdermal fentanyl patches (Fentanyl ratiopharm, Ratiopharm, Ulm, Germany) at the dose rate of 2 µg/kg/h applied to the ewe's antebrachium prior to surgery.

Experimental Protocol

After a 4-day recovery period at 124-134 gestational days, general anaesthesia was induced with a single bolus of propofol and maintained with isoflurane only. Isoflurane concentration was titrated to keep the ewe's heart rate and blood pressure normal and allow for ultrasound imaging without discomfort while minimising the physiologic alterations associated with its use. Prior to induction each ewe was prehydrated with 1 liter of Ringer's lactate solution, followed by a fixed infusion of lactated Ringer at the rate of 200 ml/h throughout the experiment. A 16-gauge polyurethane catheter was inserted into the maternal femoral artery in order to measure maternal arterial blood pressure and to obtain arterial blood gas samples. The ewe was placed supine with a right lateral tilt and allowed to stabilise for 30 minutes before the baseline measurements were taken. Thereafter, the main pulmonary artery occluder was inflated with saline until resistance was met. Complete occlusion was confirmed by colour Doppler ultrasonography, with no blood flow across the occluder. Ultrasonographic data, identical to baseline measurements, were obtained 15 and 60 minutes after the main pulmonary artery occlusion. After the 60-minute occlusion data were collected, the main pulmonary artery occluder was completely deflated to restore the main pulmonary artery blood flow. The last set of ultrasonographic measurements was taken 15 minutes after the main

pulmonary artery occluder was released. At each phase, the ultrasonographic data acquisition took approximately 15-20 minutes and the data were collected in random order. The ultrasonographic data were stored and analysed afterwards in a blind manner. At the end of the experiment, the fetus and ewe were killed with an intravenous overdose (100mg/kg) of pentobarbital sodium (Mebumat vet, Orion Oyj, Espoo, Finland), and fetal weight was determined.

Monitoring protocol

Fetal and maternal blood pressures were continuously monitored with disposable pressure transducers (DT-XX, Ohmeda, Hatfield, UK). Fetal blood pressures were referenced to intra-amniotic pressure. Heart rates were determined from the arterial pressure waveforms. Fetal electrocardiogram leads were connected to the ultrasound equipment. Maternal and fetal blood gas values were corrected to 39°C and analysed at each study point using an Abbot i-Stat 1 arterial blood gas analyser (i-Stat, East Windsor, NJ, USA).

Ultrasonographic measurements were taken by a single investigator (J.R.) using a Vivid 7 Dimension ultrasound system (GE Vingmed Ultrasound, Horten, Norway) with a 10 MHz phased-array transducer. Pulmonary and aortic valve diameters were measured and their cross-sectional areas were calculated. Blood flow velocity waveforms across the pulmonary and aortic valves were obtained with pulsed Doppler. The angle of insonation was kept at < 15 °. Volume blood flows across the pulmonary (RVCO) and aortic (LVCO) valves were calculated (Rasanen, Wood, Weiner & Huhta, 1996). Previous fetal sheep studies have shown that the proportion of pulmonary volume blood flow (Q_P) of the CCO is about 8 % at near term gestation (Rudolph & Heymann, 1970). This estimate was used to calculate fetal Q_P at baseline and recovery phases. Foramen ovale volume blood flow (Q_{FO}) was determined by subtracting Q_P from LVCO at baseline and recovery phases. During main pulmonary artery

occlusion Q_{FO} equals $LVCO$. The RV and LV fractional shortenings were calculated from M-mode recordings (DeVore, Siassi & Platt, 1984).

Longitudinal velocities of the RV and LV free wall during the cardiac cycle were assessed using pulsed-wave tissue Doppler imaging. The sample volume (1–1.5 mm) was placed at the level of the atrioventricular valve annuli and aligned as parallel as possible ($<15^\circ$) to the myocardial wall. Myocardial velocities were recorded during three to six cardiac cycles at a sweep speed of 100mm/s. The frame rate was maximised. Isovolumic relaxation (IVRV), early ventricular filling (E'), atrial contraction (A'), isovolumic contraction (IVCV) and ventricular systolic peak (S') velocities were measured. The isovolumic myocardial acceleration and deceleration were calculated (Acharya et al., 2008). The isovolumic contraction (IVCT) and relaxation times (IVRT) were measured and their proportions (%) of the total cardiac cycle were calculated (Acharya et al., 2008). Global ventricular function was evaluated by the myocardial performance index ($MPI = [IVRT + IVCT]/\text{ejection time}$) (Tei, Nishimura, Seward & Tajik, 1997).

Blood flow velocity waveforms for the ductus arteriosus (DA), umbilical artery (UA), right pulmonary artery (RPA), pulmonary vein, ductus venosus (DV) and inferior vena cava (IVC) were obtained for calculation of their pulsatility index (PI) values. To estimate volume blood flow in the placenta (Q_{Plac}), umbilical venous volume blood flow was calculated (Acharya, Wilsgaard, Rosvold Berntsen, Maltau & Kiserud, 2005).

Statistical analysis

The summary measurements are presented as means and standard deviation (SD). Repeatedly measured variables were analysed using analysis of variance for repeated measurements (ANOVA). Pairwise comparisons between different time points were performed only if the overall change over time according to ANOVA was significant ($P < 0.05$). The least

significant difference adjustment for multiple comparisons was used and if statistical significance was reached, mean differences with 95% confidence intervals (CI) were calculated. Two-tailed *P*-values were used. All analyses were performed using the SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Maternal heart rate, arterial blood pressure, blood gas values and lactate concentration remained within normal physiologic range during the entire experiment (data not shown). Maternal mean (SD) end tidal isoflurane concentration was 1.3 (0.2) % at baseline, 1.5 (0.3) % at 15 min of occlusion, 1.4 (0.4) % at 60 min of occlusion and 1.4 (0.3) % after the occlusion was released ($p = 0.247$). Fetal mean (SD) weight was 2093 (439) g. Fetal invasively measured parameters are shown in Table 1. Fetal ascending aorta blood pressure was stable throughout the experiment. Central venous pressure was higher (mean difference 2.0, 95% CI 0.8 to 3.2) during the occlusion than after it was released. Fetal pH was lower (mean difference 0.07, 95% CI 0.05 to 0.09) and $p\text{CO}_2$ higher (mean difference 1.2, 95% CI 0.4 to 2.1 kPa) at 60 minutes of occlusion than at baseline. Fetal $p\text{O}_2$, base excess and lactate concentrations did not change statistically significantly during the entire experiment.

During main pulmonary artery occlusion, no retrograde blood flow across the DA or blood flow in the pulmonary circulation could be identified by colour Doppler ultrasound. At 15 minutes of occlusion fetal heart rate (mean difference 31, 95% CI 6 to 55 to bpm) and LVCO (mean difference 147, 95% CI 54 to 239 ml/min) were greater than at baseline (Table 2, Figure 2). However, at 60 minutes of occlusion fetal heart rate and LVCO did not differ statistically significantly from baseline values. After the occlusion was released LVCO was lower than at baseline (mean difference 89, 95% CI 44 to 133 ml/min) or during the occlusion (mean difference at 15 min of occlusion 235, 95% CI 122 to 348 ml/min). Main

pulmonary artery occlusion increased Q_{FO} (mean difference at 15 min of occlusion 268, 95% CI 100 to 436 ml/min). However, the increase in Q_{FO} could only compensate for the lack of Q_P , because LV stroke volume did not change statistically significantly during the entire experiment. During the occlusion, CCO decreased about 50% from baseline values. After the occlusion was released, CCO remained lower than at baseline (mean difference 680, 95% CI 356 to 1005 ml/min). In addition, RV stroke volume (mean difference 3.97, 95% CI 2.04 to 5.91 ml), RVCO (mean difference 592, 95% CI 299 to 885 ml/min) and Q_P (mean difference 54, 95% CI 29 to 80 ml/min) were lower after the release of occlusion than at baseline (Table 2, Figure 2).

A rise in RV afterload by main pulmonary artery occlusion decreased both RV IVCV (mean difference at 15 min of occlusion 1.84, 95% 0.15 to 3.54 cm/s) and its acceleration (mean difference 1.71, 95% CI 0.29 to 3.12 m/s²), as well as IVRV (mean difference 3.25, 95% CI 2.51 to 3.99 cm/s) and its deceleration (mean difference 3.51, 95% CI 2.91 to 4.12 m/s²) (Table 3). In addition, E' (mean difference at 60 min of occlusion 3.82, 95% CI 2.04 to 5.61 cm/s) and S' (mean difference 3.31, 95% CI 1.11 to 5.52 cm/s) were lower than at baseline. Both IVRT% (mean difference at 60 min of occlusion 5.4, 95% CI 1.7 to 9.1 %) and MPI (mean difference 17.51, 95% CI 8.44 to 26.59) increased. None of these parameters were restored to baseline level after the occluder was released. However, A' and IVCT% did not change statistically significantly over the experiment ($p = 0.053$).

During main pulmonary artery occlusion, LV IVCV increased (mean difference at 15 min of occlusion 2.59, 95% CI 0.74 to 4.45 cm/s) (Table 4). After the occluder was released, it was comparable to baseline values. A' was greater during the occlusion (mean difference at 15 min of occlusion 3.83, 95% CI 1.52 to 6.14 cm/s) and after main pulmonary artery occlusion was released (mean difference 2.85, 95% CI 0.75 to 4.96 cm/s) when compared to baseline values. Furthermore, IVCT% increased (mean difference at 60 min of occlusion 3.5, 95% CI

1.2 to 5.8 %) and IVCV acceleration decreased (mean difference 1.20, 95% CI 0.16 to 2.24 m/s²) over the experiment and did not return to baseline after the occluder was released (mean difference 1.00, 95% CI 0.09 to 1.91 m/s²). No statistically significant changes in E', S', IVRV and its deceleration, as well as in IVRT% and MPI were found during the experiment.

Right ventricular fractional shortening decreased (mean difference at 60 min of occlusion 36.3, 95% CI 24.7 to 47.9 %) and did not show any recovery to baseline level after the occluder was released (mean difference 25.1, 95% CI 15.6 to 34.6 %) (Table 5). On the other hand, LV fractional shortening increased during the occlusion (mean difference at 60 min of occlusion 13.2, 95% CI 4.0 to 22.4 %), and it returned back to baseline level after the release of occluder.

In the fetal venous circulation, DV (mean difference at 60 min of occlusion 1.54, 95% CI 0.53 to 2.55) and IVC PI (mean difference 6.37, 95% CI 1.59 to 11.14) values increased during main pulmonary artery occlusion, and DV PI values remained higher also after the occlusion was released when compared to baseline (mean difference 0.94, 95% CI 0.17 to 1.71) (Table 6). After the release of occlusion, both RPA (mean difference 20.16, 95% CI 3.27 to 37.05) and pulmonary vein PI (mean difference 8.52, 95% CI 3.62 to 13.41) values were greater than at baseline, while DA PI did not differ statistically significantly from baseline. In the placental circulation, UA PI values decreased (mean difference at 15 min of occlusion 0.24, 95% CI 0.11 to 0.38) during the occlusion. After the occlusion was released, UA PI values returned towards the baseline. However, Q_{Plac} did not change statistically significantly over the experiment.

Discussion

As we hypothesised, main pulmonary artery occlusion significantly increased LVCO.

However, this increase was only about 20% from baseline values, consequently causing about 50% drop in CCO. Most interestingly, LV stroke volume did not increase during the occlusion, and a rise in LVCO was attributable to increased heart rate. Nevertheless, fetal arterial blood pressure remained stable, while central venous pressure was higher during the occlusion than after it was released. Unexpectedly, signs of LV systolic dysfunction developed while diastolic function was preserved. In the RV, a sudden increase in afterload immediately led to severe systolic and diastolic dysfunction. Fetal cardiac functional abnormalities persisted during the recovery period. Even though fetal pO_2 was maintained, a rise in pCO_2 suggested placental perfusion disturbance during the occlusion. Altogether, our findings support the concept that FO has a limited capacity to increase its volume blood flow.

During the main pulmonary artery occlusion, LV preload was entirely dependent on volume blood flow across FO, because we could not detect any retrograde blood flow across DA. It is obvious that there was a slight increase in FO volume blood flow, because LV stroke volume was maintained during the occlusion. A rise in LVCO was attributable to increased fetal heart rate. Although the Frank-Starling mechanism is functional in the fetal heart (Kirkpatrick, Pitlick, Naliboff & Friedman, 1976), the fetal ventricles seem to operate near the plateau of their function curves and have limited capacity to respond to volume loading by increasing stroke volume (Reller et al., 1987; Thornburg & Morton, 1983, Thornburg & Morton 1986). Furthermore, tachycardia shortens LV filling time and may thus decrease stroke volume (Anderson, Glick, Killam & Mainwaring, 1986). In the present study, the lack of an increase in LV stroke volume could be caused by either an inability of LV to increase its stroke volume or a limited capacity of FO to increase its volume blood flow. In a previous study with DA occlusion, we found a significant increase in LV stroke volume and LVCO that was

caused by increased Q_p . No change in FO volume blood flow was noted (Hashima et al., 2015). Furthermore, the percentage increase (40%) in LVCO was greater than in the present study. These observations suggest that the fetal LV can increase its stroke volume in response to volume loading. In addition, previous experimental work has suggested that FO blood flow cannot fully compensate for impaired pulmonary venous return (Erkinaro et al., 2007; Erkinaro et al., 2013). All these findings suggest that in fetal sheep FO volume blood flow is close to its maximum capacity at near term gestation.

Increased heart rate during the occlusion was most likely associated either with Bainbridge reflex due to atrial stretch or with a chronotropic effect induced by circulating catecholamines released in excess in response to haemodynamic stress, or both. Norepinephrine-induced peripheral vasoconstriction could explain the stable fetal arterial pressures during a remarkable decrease in CCO.

Under normal physiologic conditions, the kinetic energy of blood in the IVC is a more important determinant of FO blood flow than the pressure gradient between the two atria or that between the IVC and the left atrium (Anderson et al., 1981; Anderson et al., 1985). There are two distinct blood flow streams within the intrathoracic portion of the IVC: the high velocity DV stream, which predominantly flows through the FO, and the low velocity caudal IVC stream, which is preferentially directed into the right atrium (Schmidt, Silverman & Rudolph, 1996). In the present study, pulsatility increased in the IVC and DV, most likely reflecting elevated RV end-diastolic pressure (Hecher, Campbell, Doyle, Harrington & Nicolaides, 1995). In addition, severe cardiac dysfunction has been shown to constrain central venous blood velocity even during ventricular systole (Ghio et al., 2001). Increased pulsatility in the DV and IVC blood flow velocity further confirm that FO volume blood flow could not be substantially increased.

Tissue-Doppler derived cardiac functional parameters showed that LV A' velocity increased during the main pulmonary occlusion. Augmented left atrial contraction was most likely caused by a rise in circulating catecholamines. We observed a decrease in IVCV acceleration during prolonged main pulmonary artery occlusion. Because IVCV acceleration is a load-independent index of myocardial contractility (Vogel et al., 2002), this indicates that LV systolic dysfunction developed over the experiment. However, IVRV and its deceleration were not affected, suggesting well preserved LV diastolic function. The declining LV systolic function without signs of LV diastolic dysfunction was in contrast with our expectations. We anticipated that increased levels of circulating catecholamines during the occlusion would enhance LV contractility. In addition, increased atrial pressure is known to rapidly induce atrial natriuretic peptide secretion in fetal sheep (Jaekle, Sheikh, Berry, Washburn & Rose, 1995), which could potentially have a positive effect on both systolic and diastolic function (Ozawa et al., 2015). As expected, a severe increase in RV afterload resulted in significant RV systolic and diastolic dysfunction with increased RV dimensions and a leftward shift of the interventricular septum during systole, as verified by negative RVFS values during the occlusion. Consequently, we propose that the concomitant RV dysfunction and altered interventricular septal movement, through ventricular interdependence, is at least one possible mechanism for the decrease in LV contractility in the present study. After the main pulmonary artery occlusion was released, RV systolic and diastolic dysfunction, as well as LV systolic dysfunction persisted. The most likely explanation is that increased right ventricular end-diastolic pressure during main pulmonary artery occlusion could impair coronary artery blood flow and limit the oxygen delivery to the subendocardial area of the myocardium.

Umbilicoplacental circulation lacks significant autoregulation and is directly proportional to perfusion pressure (Berman, Goodlin, Heymann & Rudolph, 1976). A 50 % drop in CCO during the main pulmonary artery occlusion deteriorated placental circulation to some extent. Although the decrease in placental volume blood flow was not statistically significant, it

resulted in derangement of placental gas exchange, since the decrease in fetal pH was associated with respiratory acidemia without a metabolic component. Umbilical artery PI values decreased during the occlusion demonstrating that UA blood flow velocity waveform is not a direct measure of placental vascular resistance, rather it reflects the number of tertiary villous arterioles (Giles, Trudinger & Baird, 1985). Our findings demonstrate that sufficient placental perfusion for fetal survival can be maintained by fetal compensatory mechanisms that aim to direct blood flow and perfusion to vital fetal organs.

The present study was designed to investigate the capability of FO to increase its volume blood flow when main pulmonary artery is obstructed. We recognise that an acute complete occlusion of the main pulmonary artery is not a physiologic event. However, fetuses with right ventricular outflow tract obstruction or tricuspid atresia depend mainly on blood flow across the FO. In human fetuses with pulmonary outflow tract obstruction, retrograde blood flow in DA that is directed to the pulmonary circulation helps to support LVCO (Peyvandi et al., 2014). It is known that during the last trimester, human fetal pulmonary circulation becomes responsive to changes in fetal oxygenation and in normal physiologic environment fetal pulmonary vascular resistance increases thus limiting pulmonary venous return to the left atrium (Rasanen et al., 1998; Rasanen, Wood, Weiner, Ludomirski & Huhta, 1996). If the fetus becomes hypoxemic, vasoconstriction in the pulmonary circulation further reduces lung blood flow. Thus, in the fetus with right ventricular outflow tract obstruction or tricuspid atresia, Q_{FO} becomes a critical factor in order to maintain systemic blood flow and perfusion. Our results have significant clinical value. Fetuses with pulmonary outflow tract obstruction or tricuspid atresia could be at higher risk for intrauterine demise, if hypoxemia develops, especially during the last trimester of pregnancy.

There are limitations in our study. We acknowledge that the sample size is relatively small which may limit the power of our study. In this kind of experimental study with completely

eliminating cardiac output from one of the ventricles, it is virtually impossible to make any reliable power calculations. The sample size is comparable to that of previous experiments on large laboratory animals. The fetuses underwent surgical procedures that may constitute a major stress. Yet, the recovery period after surgery should be long enough for proper recovery of fetal myocardial function (De Muylder, Fouron, Bard & Urfer, 1983). The experiments were performed under general anaesthesia that could modify fetal cardiovascular adaptation. It has been shown that cardiovascular system of the new-born lamb can increase oxygen delivery in response to hypoxemic stress during isoflurane anaesthesia. Therefore, at reasonable anaesthetic depth, and without myocardial or peripheral cardiovascular disease, the newborn lamb can coordinate neural, endocrine, and local tissue responses to increase cardiovascular performance in response to hypoxemia (Brett, Teitel, Heymann & Rudolph, 1989). There are some differences in cardiovascular physiology and anatomy between human and sheep fetuses. However, sheep experiments have provided invaluable information on fetal hemodynamic regulation. Previous Doppler ultrasonographic studies suggest that the phasic flow events associated with the cardiac cycle are comparable in human and sheep fetuses (Kiserud, Eik-Nes, Blaas & Hellevik, 1992; Schmidt, Silverman & Rudolph, 1996). In addition, validation studies in sheep fetuses have proven that invasive and Doppler echocardiographic volume blood flow calculations correlate well (Schmidt, Di Tommaso, Silverman & Rudolph, 1991). The intraobserver variabilities of Doppler ultrasonographic parameters of fetal sheep cardiovascular haemodynamics and tissue Doppler derived indices are comparable to those found in human fetuses during the second half of pregnancy (Bernard et al., 2012; Rasanen et al., 1998). Finally, FO volume blood flow was calculated by subtracting Q_p from LVCO at baseline and recovery phases. Unfortunately, direct measurement of volume blood flow across the FO is impossible.

In conclusion, complete main pulmonary artery occlusion led to about 20% increase in LVCO and about 50% reduction in CCO. During the occlusion LV stroke volume did not change. A rise in LVCO was related to an increase in fetal heart rate. Fetal arterial blood pressure was maintained during the occlusion, while central venous pressure was higher during the occlusion than after it was released. During main pulmonary artery occlusion, LV systolic dysfunction developed while diastolic function was preserved. Severe RV dysfunction was reflected as increased pulsatility in systemic venous blood flow patterns. Fetal pO_2 was maintained, however, a rise in pCO_2 suggested placental perfusion disturbance during the occlusion. Altogether, our findings show that FO has a limited capacity to increase its volume blood flow.

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Additional information**Competing interests**

The authors report no conflict of interest.

Author contributions

Conception and design of the experiments: L.E.D., A.R.H., G.A. and J.R. Acquisition, analysis, or interpretation of data for the work: all authors. Drafting the work or revising it critically for important intellectual content: all authors. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Table 1. Fetal arterial blood gas and blood pressure measurements

	Baseline	PaO 15 minutes	PaO 60 minutes	PaO release	P-value for time
pH	7.33 (0.04)	7.30 (0.02)	7.26 (0.04)*	7.24 (0.09) *	0.043
pO ₂ (kPa)	2.6 (0.6)	2.3 (0.4)	2.1 (0.6)	2.3 (0.5)	0.176
pCO ₂ (kPa)	6.3 (0.6)	6.9 (0.9)	7.5 (0.8)*†	7.1 (1.3)	0.033
Base excess (mmol/l)	-0.9 (2.9)	-0.6 (3.2)	-1.4 (4.2)	-3.3 (6.8)	0.265
Lactate (mmol/l)	3.36 (1.33)	3.07 (1.16)	3.40 (1.69)	3.91 (2.28)	0.199
Ascending aorta blood pressure		46 (5)	45 (4)	47 (4)	0.719

Systolic (mmHg)	47 (6)				
Mean (mmHg)	38 (3)	37 (4)	37 (3)	38 (5)	0.928
Diastolic (mmHg)	32 (3)	32 (4)	32 (3)	32 (4)	0.996
CVP (mmHg)	4 (3)	6 (3)	5 (3)	4 (3) ‡	0.012

Values are means with SD in parentheses; N=7. Abbreviations: *PaO*, main pulmonary artery occlusion; *CVP*, central venous pressure.

* different than Baseline, $P < 0.05$

† different than preceding phase, $P < 0.05$

‡ different than *PaO* 15minutes phase, $P < 0.05$

Table 2. Fetal Cardiovascular Parameters

	Baseline	PaO 15 minutes	PaO 60 minutes	PaO release	P-value for time
FHR (bpm)	158 (7)	188 (23)*	177 (20)	160 (18)	0.022
LVSF (ml)	4.14 (1.40)	4.14 (1.47)	3.95 (1.48)	3.53 (1.04)	0.067
LVCO (ml/min)	629 (198)	776 (283)*	720 (273)	541 (172)*†	0.0001
RVSF (ml)	5.85 (1.75)	0	0	1.88 (1.37)*	0.002
RVCO (ml/min)	895 (259)	0	0	303 (229)*	0.003
CCO (ml/min)	1524 (341)	776 (283)*	720 (273)*	844 (290)*	0.0001
Q _p (ml/min)	122 (27)	0	0	68 (23)*	0.002
Q _{FO} (ml/min)	507 (181)	776 (283)*	720 (273)*	473 (159)†	0.001

Values are means with SD in parentheses; N= 7. Abbreviations: *PaO*, main pulmonary artery occlusion; *FHR*, fetal heart rate; *LVSF*, left ventricular stroke volume; *LVCO*, left ventricular cardiac

output; *RVSV*, right ventricular stroke volume; *RVCO*, right ventricular cardiac output; *CCO*, combined cardiac output; Q_p , pulmonary volume blood flow; Q_{FO} , foramen ovale volume blood flow.

* different than Baseline, $P < 0.05$

† different than preceding phase, $P < 0.05$

Table 3. Fetal right ventricular tissue Doppler parameters

	Baseline	PaO 15 minutes	PaO 60 minutes	PaO release	P-value for time
E' (cm/s)	5.71 (1.10)	2.86 (2.07)*	1.88 (1.51)*	2.47 (1.72)*	0.002
A' (cm/s)	9.69 (1.74)	11.15 (3.91)	9.72 (1.72)	9.08 (1.41)	0.373
S' (cm/s)	8.38 (2.11)	5.78 (1.46)*	5.07 (0.75)*	5.08 (0.98)*	0.001
IVCV (cm/s)	5.31 (1.35)	3.46 (0.94)*	3.27 (0.96)*	2.93 (0.54)*	0.001
IVCV acceleration (m/s ²)	5.39 (1.10)	3.68 (1.15)*	3.60 (1.16)*	3.42 (1.14)*	0.002
IVRV (cm/s)	3.25 (0.80)	0*	0.24 (0.64)*	1.86 (0.27)*†	0.0001
IVRV deceleration (m/s ²)	3.51 (0.66)	0*	0.16 (0.43)*	2.14 (0.55)*†	0.0001
IVCT (%)	6.8 (1.4)	10.5 (5.4)	11.2 (2.8)	9.9 (3.4)	0.078
IVRT (%)	11.9 (2.5)	16.9 (2.8)*	17.3 (3.5)*	18.2 (2.3)*	0.0001
MPI	0.45 (0.07)	0.61 (0.25)	0.63 (0.15)*	0.73 (0.25)*	0.004

Values are means with SD in parentheses; N=7. Abbreviations: *PaO*, main pulmonary artery occlusion; *E'*, early ventricular filling velocity; *A'*, atrial contraction velocity; *S'*, ventricular systolic peak velocity; *IVCV*, isovolumic contraction velocity; *IVRV*, isovolumic relaxation velocity; *IVCT*, isovolumic contraction time; *IVRT*, isovolumic relaxation time; *MPI*, myocardial performance index.

* different than Baseline, $P < 0.05$

† different than preceding phase, $P < 0.05$

Table 4. Fetal left ventricular tissue Doppler parameters

	Baseline	PaO 15 minutes	PaO 60 minutes	PaO release	P-value for time
E' (cm/s)	8.05 (2.26)	5.33 (2.64)	5.28 (2.88)	6.32 (2.64)	0.7
A' (cm/s)	11.73 (1.91)	15.57 (3.13)*	16.41 (3.85)*	14.59 (3.05)*	0.03
S' (cm/s)	7.07 (1.13)	7.64 (1.96)	7.01 (1.78)	6.62 (1.24)	0.116
IVCV (cm/s)	5.72 (1.39)	8.32 (2.49)*	8.02 (3.05)	6.66 (1.70)	0.017
IVCV acceleration (m/s ²)	4.65 (1.04)	4.00 (0.98)	3.45 (0.84)*	3.65 (1.18)*	0.043
IVRV (cm/s)	2.70 (0.37)	1.86 (1.32)	1.63 (1.13)	2.38 (0.34)	0.131
IVRV deceleration (m/s ²)	3.37 (0.82)	2.30 (1.67)	1.90 (1.36)	2.80 (0.58)	0.076
IVCT (%)	7.2 (0.9)	10.7 (1.3)*	10.7 (2.3)*	10.3 (2.9)*	0.006
IVRT (%)	11.7 (2.0)	13.4 (2.2)	13.4 (2.6)	13.8 (1.4)	0.212
MPI	0.43 (0.04)	0.57 (0.13)	0.53 (0.13)	0.50 (0.22)	0.353

Values are means with SD in parentheses; N=7. Abbreviations: *PaO*, main pulmonary artery occlusion; *E'*, early ventricular filling velocity; *A'*, atrial contraction velocity; *S'*, ventricular systolic peak velocity; *IVCV*, isovolumic contraction velocity; *IVRV*, isovolumic relaxation velocity; *IVCT*, isovolumic contraction time; *IVRT*, isovolumic relaxation time; *MPI*, myocardial performance index.

* different than Baseline, $P < 0.05$

Table 5. Fetal cardiac dimensions

	Baseline	PaO 15 minutes	PaO 60 minutes	PaO release	P-value for time
Left Ventricle					
Diastole (cm)	1.25 (0.15)	1.22 (0.11)	1.17 (0.17)	1.21 (0.18)	0.654
Systole (cm)	0.80 (0.10)	0.62 (0.19)	0.59 (0.11)*	0.75 (0.14)	0.029
FS (%)	35.8 (6.2)	49.0 (12.1)	49.0 (9.6)*	37.6 (8.3)	0.024
Right Ventricle					
Diastole (cm)	1.14 (0.16)	1.37 (0.26)	1.41 (0.19)*	1.33 (0.19)*	0.001
Systole (cm)	0.81 (0.22)	1.42 (0.30)*	1.51 (0.25)*	1.27 (0.25)*†	0.001
FS (%)	30.0 (10.4)	-4.0 (13.4)*	-6.3 (7.4)*	4.9 (6.5)*†	0.0001

Values are means with SD in parentheses; N=7. Abbreviations: *PaO*, main pulmonary artery occlusion; *FS*, fractional shortening.

* different than Baseline, $P < 0.05$

† different than preceding phase, $P < 0.05$

Table 6. Fetal peripheral haemodynamics and placental volume blood flow

	Baseline	PaO 15 minutes	PaO 60 minutes	PaO release	P-value for time
Pulsatility Index					
DA	2.71 (0.86)	-	-	4.56 (3.14)	0.286
RPA	4.78 (1.85)	-	-	24.94 (18.11)*	0.027
P _{vein}	1.23 (0.26)	-	-	9.75 (4.60)*	0.007
UA	1.41 (0.25)	1.17 (0.17)*	1.26 (0.34)*	1.28 (0.33)	0.017
IVC	1.95 (1.24)	10.80 (9.57)	8.32 (4.88)*	4.93 (4.31)	0.023
DV	0.86 (0.27)	2.14 (0.91)*	2.40 (0.96)*	1.80 (0.70)*	0.003
Q _{Plac} (ml/min)	156 (66)	88 (27)	105 (41)	125 (69)	0.165

Values are means with SD in parentheses; N=7. Abbreviations: *PaO*, main pulmonary artery occlusion; *DA*, ductus arteriosus; *RPA*, right pulmonary artery; *P_{vein}*, pulmonary vein; *UA*, umbilical artery; *IVC*, inferior vena cava; *DV*, ductus venosus; *Q_{Plac}*, placental volume blood flow.

* different than Baseline, $P < 0.05$

Figure Legends

Figure 1. Location of the main pulmonary artery occluder.

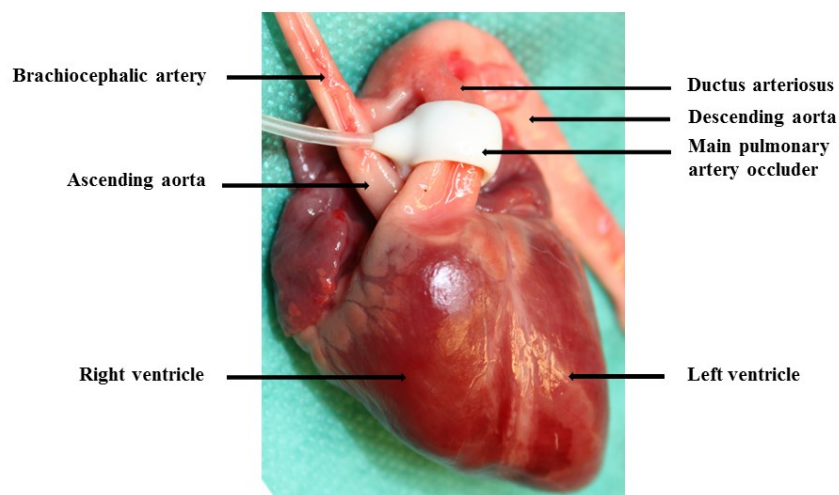


Figure 2. Fetal combined (CCO), left ventricular (LVCO) and right ventricular (RVCO) cardiac outputs, pulmonary (Q_p) and foramen ovale (Q_{fo}) volume blood flows, and fetal heart rate (FHR) during the main pulmonary artery (PA) occlusion.

